APPLICATION OF INFRARED THERMOGRAPHY IN RABBIT ORTHOPAEDIC MODELS

Ioan HUTU[1,3], Irina PATRAS[1,3], Diana GHERGHEL[3], Bianca LUNGU[3], Calin MIRCU[2,3*]
Faculty of Veterinary Medicine, Banat University of Agricultural Science and Veterinary Medicine *King Michael I of Romania* – Timisoara, 119th Aradului Street, 300645, TM - RO
[3] Experimental Unit from *Horia Cernescu* Research Experimental Units, Banat University of Agricultural Science and Veterinary Medicine *King Michael I of Romania* – Timisoara
*Correspondent author e-mail address: calinmircu@usab-tm.ro

Abstract
The rectal or internal temperature (BT) is a reference method for body temperature. BT and ear temperature (BT_{ear}) were recorded in rabbit orthopaedic experimental model - White New Zeeland rabbits (N = 14), for a six day post-surgery period. Ear (BT_{ear}) temperature measured with infrared thermography (IRT) camera was compared with rectal body temperature (BT) measured with digital thermometer. Each BT_{ear} and BT methods were studied by analysis of variance and for BT classes such as: hypothermia ($BT_h \geq$ than 38,5°C), normothermia (BT_n) and hyperthermia or fever ($BT_f \geq$ 40,0 °C). Mean differences, linear regression and Pearson correlation were analysed. BT_{ear} was positively correlated with rectal temperature (BT); r=+0.579 at p <0.001. The regression equation model was statistically acceptable (p<0.001) and value of internal body temperature can be estimated on ITR measurements by relation: BT (°C) = 25.498 + BT_{ear} x 0.361 with R^2=0.336. This study demonstrates that IRT technology, a passive and non-contact technology can be effectively used for estimating BT changes in rabbits.
Keywords: *infrared thermography, body temperature, ear temperature, rabbit.*

Introduction

Experimental infrastructure of *Horia Cernescu* Research Unit is a research infrastructure which is running projects under Authorization no. 535 / 19th of May 2016. Animal orthopaedic models are considered to produce high levels of pain, suffering or distress and any manipulation can increase those. Even the rectal temperature measurement is a simple intervention which involves manipulation and restrain – and can produce stress and pain for the animals.

The infrared thermography (IRT) is enabling sensitive and alternative methods of measuring body temperatures (BTs) and it can be used to identify both hypothermia and hyperthermia or fever (8). Non-contact IRT is a promising technology that recently has been reviewed for its use in veterinary applications (6,7). Among other applications, non-contact IRT has been examined to successfully detect inflammatory conditions or fever in horses, cows or ponies (1,2 & 5).

Specific target of the study was to establish associations between *internal (rectal) body temperature (BT)* measured by digital thermometer and ear temperature (*BT_{ear}*) assessed by non-contact infrared thermography (FLIR infrared camera) in order to reduce the manipulation of animal in future projects (e.g. developed with orthopaedic rabbit models).

Materials and methods ***Animals and data collection -*** Two groups out of five[1], which forms the sample, (14 out of 35 White New Zeeland rabbits) were used for both external and internal temperature monitoring six days after surgery intervention.

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[1]The animal are coming from a study of the regenerative potential of mesenchymal stem cells at the level of meniscal lesion. The approved APS divided the rabbits into 5 groups (BMAC - Bone Marrow Aspirate Concentrate, PRP - Platelet-Rich Plasma, AC - Agili C, CD - Chondrotissue and C - Control), each group had 7 animals.

For refinement reason, the telemetry was used for identification - ID microchip was implanted s.c. to each rabbit. The rabbits had 6 months of age and 3,562.07±68.16 g weight (X ± sx), measured ~~h~~ one day before creating animal orthopaedic models with knee trauma (causing meniscus and cartilage lesions). All animals were clinically healthy and normotherms - 38.5-40.0 ^{o}C.

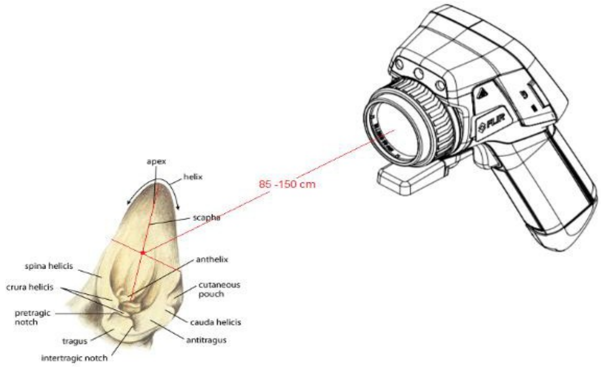


Figure 1: Election point (spot) for measuring rabbit ear temperature by infrared camera.
Surce of imagines: Anatomy of rabbit ear, ilustration by Gheoghe Constantinescu for Merk, (11) & FLIR® (10).

Body temperature was determined using two measurement locations. Internal body temperature (BT) was measured by insertion of a digital thermometer (*Flex Temp Smart, OMRON Healthcare Co., Ltd., Kyoto, Japan* with accuracy ± 0,1°C between 32,0°C to 42,0°C) approximately 1 cm into the rectum to acquire an automated reading upon pressing the measurement button. Ear temperature (BT_{ear}) was detected by high resolution infrared detection camera *(FLIR E50 Multi-Spectral Dynamic Imaging, MSX®, Wilsonville, Oregon, USA)* with 50mK (0.05°C) thermal sensitivity by spot and thermal imagines. The infrared images were obtained by manually focus, upon pressing the laser for guidance of the spot and recording into camera. The election point was in the ear scapha, on the axis of auricular pavilion at the half distance between apex and tragus (see figure no. 1 and 2). The infrared image was automatically adjusted.

Housing and feeding. The rabbits were kept in a three level *Techniplast® X-type cage, L x l x h* = 784 x 820 x 1830 mm and 4.264 cm^2 space. The walls and floors are made of transparent (side panels) or opaque polycarbonate (rear panels, discontinuous floor and trash and purine trays). In the rabbits' compartment, the environment temperature and humidity were continuously monitored (every half an hour) by multi-functional wireless digital device *Weather Station PCE-FWS 20*. The environmental temperature was 21.07±0.2 and the study do not sustain significant differences between days of measurements. The value of air speed in the room of rabbit was 0.01 m/s (one time per day measurements).

Figure 2: Measuring the rabbit ear temperature by IRT.
The position of the technician involved in IRT non-contact temperature prelevation (left) and laser point of the FLIR camera on the ear scapha, the election point. The door of the cage close (on the left) or open (in the right). In the right picture the animal went to the back of the cage to avoid human manipulation or restrain.
Surce: UEX Media, Experimental Unit, 2018.

Adult rabbit consumed daily 160-180 g of pelleted feed with ~~the~~ digestibility up to 65%. The metabolic energy density of feed was 1980±50 kcal / kg. The calories were coming from protein (23%), fat (10%) and carbohydrates (67%).

Statistical Analysis: *Paired t tests* and *Bland–Altman plot* analysis were used to assess differences in mean values for BT's measured by IRT versus the reference method (rectal temperature). Analysis of associations with several factors or variables (BT classes by BT_{ear}, weight, environmental temperature) were performed based on *Variance Analysis* (ANOVA), *Pearson correlation* and *regression* with *IBM® SPSS® Statistics* software, product of IBM Corporation, 2015. Significance was determined at a value of $\alpha = 0.05$.

Results

Body internal or rectal temperature (BT) was measured on 14 animals for seven consecutively days at the same hour - 22:00 p.m. The average and standard error (X±sx) of internal body temperature measured by digital thermometry was 39.03±0.7 ºC. During the monitoring period, 10 rabbits were in hypothermia, in the next several hours or in first day after surgical procedures – the temperature was 37.93±0.16 and 5 rabbits had fever – 41.04±0.29 ºC. The study could not associated the classes of BT with body weight (F=1,789 at p=0.173), day of study or room temperature.

Ear temperature (BT_{ear} – figure no. 3) for the same 14 rabbits was 37.50±0.12 ^{o}C, with 1.53±0.10^{o}C less then BT – the difference between internal and BT_{ear} temperature was significant (t=10.20, at p<0.001). The values of BT_{ear} (X±sx) for the rabbits with hypothermia BT class was: 35.73±0.40 ºC (with 95% CI 34.78 ÷ 36.69 ºC). For normothermic BT rabbits the ear temperature was 37.72±0.10 ºC, with 95% CI between 37.51 to 37.92 ºC.

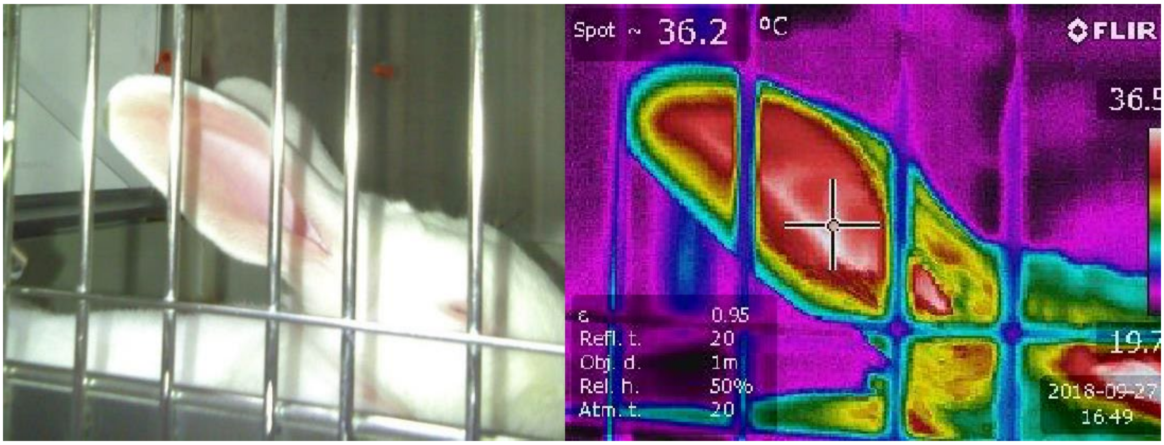
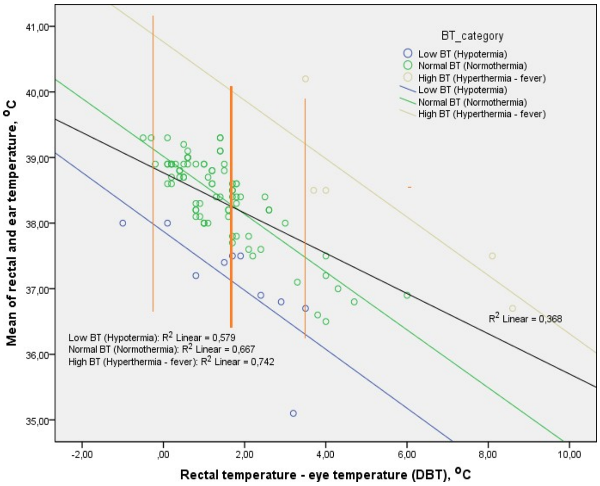


Figure 3: Clasical and thermal imagins of ear captured by thermocamera of the rabbit.
The clasical pictures of FLIR camera (left) and the thermal imaging of the same animal (right). The spot temperature of the election point was considered the ear temperature (BT_{ear}). The WNZ rabbit was in the cage – no contact between technician and animal and no door open was performed. There is a difference between a spot temperature (36.2°C) and maximum temperature of termograpy image (36.5°C).
Surce of imagines: UEX Media, Experimental Unit, 2018.

The difference (D_{BT}) and average (X_{BT}) between BT and BT_{ear} was performed for each animal and for all measurements. The average and standard deviation values X_{BT} 38.28±0.76°C and the average of difference was D_{BT} = 1.53±0.10°C (Graph no. 1). The Pearson correlation was found between BT and BT_{ear} (r = + 0.579, at p <0.001), BT and X_{BT} (r = + 0.831, at p <0.001), BT_{ear} and X_{BT} (r = + 0.934, at p <0.001), BT_{ear} and D_{BT} (r = - 0.783, at p <0.001).



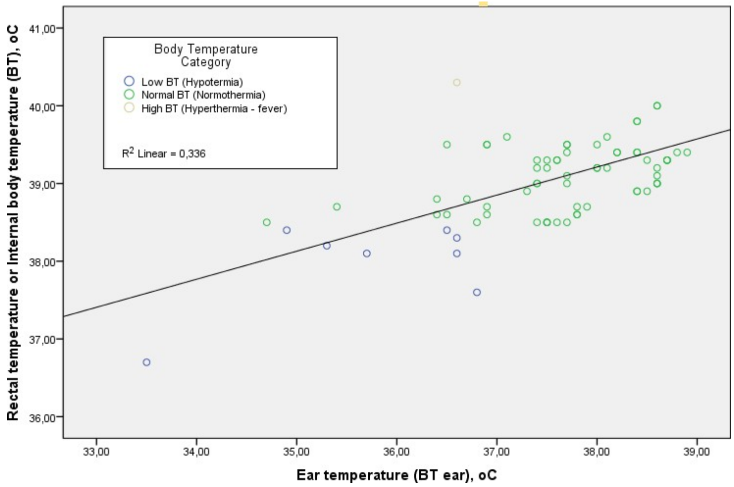
In the graph, the oblique thin lines are the lines of best fit for the data, the thick solid vertical line is the mean difference (bias), and the vertical thin lines are the 95% limits of agreement (mean difference ± 1.96 SD, respectively 1.53±1,96x0.86°C). Points located on the left to -0.16 or on the right to +3.22 on the x-axis represent an underestimation of temperature by the IFR method in comparison to rectal core body temperature as measured by digital thermometer.
The value for regression of DBT for XBT has a determination coefficient R^2 = 0.368; the values of R^2 for each classes of BT are show in the graph.

Graph 1. Bland-Altman plots of body temperature in rabbits comparing rectal (BT) and ear temperature (BT_{ear})

The linear regression (Graph no. 2) of body temperature in rabbits (BT) by spot ear temperature (BT_{ear}) is written in a relation *(1)*:

$$BT = 25.498 + 0.361 \times BT_{ear} \qquad (1)$$

($R^2 = 0.336$, t value = 11.36 at $p < 0.001$)



Graph 2. Body temperature (BT) regression by ear temperature (BT_{ear}).

Discussion

Using telemetry for ID identification (microchip) and temperature measurement (thermal camera) represent both responsibility of researcher and refinement of experimental techniques. Ear, tympanic membrane and hypothalamus share blood supply from the carotid arteries (3); past research has reported that tegument surface temperatures are correlated with body temperature (4,9). However this non-contact, fast and flexible approach gave resulted in too low, non-physiological temperatures, probably due to the isolating fur at the measuring spot. Furthermore, analysis of a single defined spot (in our case: middle or ear) on the auricular surface seems questionable for reproducible temperature measurements, since it can be strongly influenced by the isolating fur and heat accumulation in resuscitation cage used in intensive therapy, after anesthesia.

A more sophisticated telemetry device - microchip implanted s.c. - permit both animal identification and body temperature and give more accurate values of temperatures measurement.

Compared to a single spot infrared thermometer, thermography generates images that can be analyzed on a per pixel basis as if thousands of infrared spot thermometers are used simultaneously. Using thermal imaging we were quickly able to identify the ear areas as the most prominent and warmest surface of the rabbit ear scapha (Fig. 1). Thus, the IRT can be used for measurement of temperature of orthopedic rabbit model even if the coefficient of multiple determination is not very high (R^2 was 0.336) because this method reduce the restrain and animal fixation time resulted. If the value of thermal spot imaging is abnormal there can be used classical measurements. Because there is no direct contact, this fast, flexible technology contributes to decrease the risk of pain, suffering or distress in orthopedic rabbit models.

Conclusions

- The ITR, a non-contact, fast and flexible technology eliminate the restrain and manipulation of rabbit orthopaedic models. As a result, the levels of pain, suffering or distress are reduced.
- The spots of IRT imaging taken from ear (BT_{ear}) can be used to estimate the body temperature by a regression equation.
- BT_{ear} can be useful to identify both hypothermia and fever rabbits.
- Using a more sophisticated microchip for both temperature measurement and animal identification have to by experiment in a future research projects.

Ethic statement

Study protocol was designed and followed in strict accordance with the guidelines of Experimental Units under Veterinarian Authority authorization no. 002 / 2018 and was a supporting activity for the project ORTO STEM REGEN & SIHFALCAG.

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